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Carcino-Embryonic Antigens, Oestrogen Receptors and Androgen Receptors in Human Breast Tumours

Clinical evaluation of carcino-embryonic antigen, IV

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Summary: This paper, the fourth in a series devoted to the study of the clinical usefulness of estimations of carcino-embryonic antigen (CEA), describes CEA levels in extracts of metastatic breast tumours.

– Two groups can be distinguished, with CEA values higher or lower than 1.5 μg CEA per g protein. The group of tumours with a CEA level exceeding 1.5 μg/g (CEA-positive) included a significantly larger percentage of oestrogen receptor-positive tumours than the group with lower CEA levels (CEA-negative).

– It is stated that CEA-negative metastases are most likely to be found in patients who fail to respond to hormonal therapy.

– No relation was demonstrable between the presence of androgen receptors and the CEA level. All the possible permutations of CEA, oestrogen receptors and androgen receptors were encountered in the tumours examined.

Carcinoembryonale Antigene, Östrogenrezeptoren und Androgenrezeptoren bei Mammatumoren des Menschen. Klinische Bedeutung des carcinoembryonalen Antigens, IV.

Zusammenfassung: Diese vierte Arbeit einer Serie, die sich mit der klinischen Bedeutung der Bestimmung von carcinoembryonalem Antigen (CEA) befaßt, beschreibt CEA-Werte in Extrakten von Metastasen von menschlichen Mammatumoren. Die statistische Auswertung der Ergebnisse ergab zwei Gruppen mit einem Grenzwert von 1,5 μg CEA pro Gramm Eiweiß.

Ein Vergleichsstudium bezüglich der Konzentrationen von Östrogen- und Androgenrezeptoren lieferte nachfolgende Ergebnisse:

Die erste Gruppe, angedeutet als CEA negativ (mit CEA Werten unter 1,5 μg/g) enthält einen wesentlich niedrigeren Prozentsatz an Östrogenrezeptoren als die andere Gruppe. Hieraus ließ sich schließen, daß Patienten, bei denen die hormonale Therapie einen ungünstigen Effekt hat, in den meisten Fällen CEA-negative Metastasen haben. Es wurde gezeigt, daß die CEA-Bestimmung in Tumorextrakten die Messung der Östrogenrezeptoren für klinische Zwecke nicht ersetzen kann.

Es wurde nachgewiesen, daß kein Zusammenhang zwischen dem Vorkommen von Androgenrezeptoren und den CEA-Werten besteht. Offenbar besteht eine große Kombinationsmöglichkeit der obengenannten Substanzen in menschlichen Mammatumoren.

Introduction

It is now well established that patients suffering from breast cancer with oestrogen receptor-positive metastases have about a 60 percent chance of benefitting from hormonal therapy, be it additive or ablative. In patients with oestrogen receptor-negative metastases this chance is low, but still about 10 percent (1).

The question which arises in this context is whether determination of other parameters might lead to a more detailed selection of patients so that therapeutic effects could be even more reliably predicted.

Wagner et al. (2) and *Persijn* et al. (3) determined the occurrence of oestrogen receptor and androgen receptor in 24 and 51 breast tumours, respectively. In some cases

both receptors were present, in others one or the other receptor was absent, and there were cases in which both receptors were absent. Recently *Wagner & Jungblut* have published a more detailed study of this subject (4).

Botet et al. (5) compared the results of oestrogen receptor determinations in extracts of breast tumour tissue and of normal mammary tissue with those of CEA assays in the same extracts. They found an overall concordance of about 60 percent between oestrogen receptor and CEA in malignant specimens.

This paper presents the results of a study based on determinations of oestrogen receptor, androgen receptor and CEA in about 200 breast tumour tissue extracts.

Material and Methods

Assay of oestrogen receptors and androgen receptors

– Oestrogen receptors and CEA[1]) were measured in extracts of 100 benign and malignant tumours; in 80 of these androgen receptors were also determined. In another series of 156 tumours, both receptors were determined. The extracts were prepared as described in reference (6). Portions of the extracts were used immediately after preparation for receptor assays, while other portions were kept frozen until used for CEA assays. Oestrogen receptor and androgen receptor contents were measured by electrophoresis according to *Wagner* (7). The classification of receptor-negative and receptor-positive tumours was carried out by probit analysis as described in references (8) and (9).

CEA assay in extracts of breast tumours

CEA assays in tumour extracts were carried out with the aid of antiserum NKI-3 and CEA prepared by us (2SDI)[1]), the properties of which have been described elsewhere (10).

In round-bottomed plastic tubes (length 4 cm, Ø 8 mm), 50 µl extract or standard CEA solution was mixed with 20 µl NKI-3[1]), diluted about 1:60,000 with phosphate-buffered saline containing rabbit serum.

The mixture was incubated overnight at 37 °C, whereupon 20 µl [125 I]CEA (3 µg/l) was added to each tube; incubation was then continued for 5 hours at 37 °C. Antibody-bound CEA was separated from unbound CEA using the double antibody technique as described in reference (10).

For control, portions of two CEA solutions in Tris buffer (containing about 4.7 and 9.6 µg/l, respectively) were always included in the series of samples. The compositions of the Tris buffer and the phosphate-buffered saline are described in references (8) and (10) respectively.

Evaluation of the CEA assay in extracts

The sample volumes available were too small for a duplicate CEA assay as described in reference (10) (radio-immunoassay, system 2) as well as the analyses of the various receptors. The use of a reduced sample volume necessitates alteration of the procedure in order to ensure adequate sensitivity of the CEA assay. Increased sensitivity was ensured by reducing the final volume of the reaction mixture and the amount of anti-CEA antiserum added.

Figure 1 shows a standard inhibition curve which is representative for the curves obtained in our laboratory with NKI-3 in CEA assays in extracts. Since the tissue extracts were prepared with Tris buffer (8), standard inhibition curves obtained with Tris and with PBS were compared. The two curves coincided completely. The influence of tissue proteins present in extracts was also studied. Standard inhibition curves with and without extract of a benign breast tumour coincided completely.

– For assays of small quantities of CEA in tumour extract it is of importance that the CEA used in the radio-immunoassay be free from serum proteins. Experiments described in reference (10) had already indicated that this is the case. In order to achieve even greater certainty about possible contamination of 2SDI with serum proteins, an experiment was carried out in which labelled 2SDI was incubated with horse anti-human serum antiserum. Rabbit antihorse antiserum was added to precipitate the resulting complex. As demonstrated in table 1, no significant binding of labelled 2SDI to horse anti-human serum antiserum was found. The reproducibility of the determination was tested against results obtained with control solutions. For the solution with 4.4 µg/l CEA, a standard deviation of 0.52 µg/l (n = 25) was found; the standard deviation found for the solution with 9.6 µg/l was 0.99 µg/l (n = 25).

Results

Nearly equal percentages of oestrogen receptor and androgen receptor were found in both primary and metastatic breast tumours (tab. 2). The distribution shown in table 3 reveals that all four combinations can be encountered. There are tumours which contain only oestrogen receptor, only androgen receptor, or both, or neither.

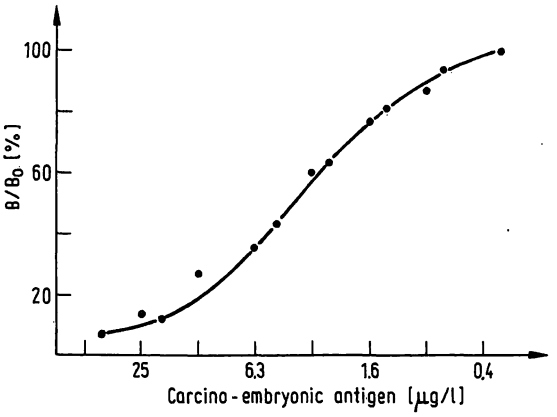

Fig. 1. Standard inhibition curve.
Antiserum: NKI-3, diluted 1/60,000.

Tab. 1. Percentage of precipitated labelled 2SDI after incubation with horse anti-human serum antiserum for 3 hours (37 °C), followed by incubation with rabbit anti-horse antiserum overnight (4 °C). FFZ = Phosphate buffer.

Rabbit anti-horse anti-serum*	Horse anti-human serum antiserum* 1:5	1:10	1:50	1:100	1:250	1:500	1:1000	FFZ
1:2	3.3	4.1	4.6	2.8	5.1	4.7	8.1	4.1
1:4	3.9	4.5	5.1	7.4	3.4	5.1	4.2	4.0
1:8	2.5	2.3	3.8	2.9	3.9	1.6	2.9	0.4
1:16	3.0	1.2	1.6	2.8	0.6	4.1	0.1	0.3
1:32	1.8	0.2	0.2	2.5	1.4	4.5	3.6	2.7

* diluted with phosphate-buffered saline.

[1]) Abbreviations:
CEA = carcino-embryonic antigen.
2SDI = CEA prepared by us.
NKI-3 = CEA antiserum.

Tab. 2. Percentages of oestrogen receptor-positive or androgen receptor-positive primary and metastatic breast tumours.

Breast cancer tissue	Receptors present	
	oestrogen	androgen
primary*	42.6	41.2
metastatic**	36.4	37.5

* n = 68
** n = 88

Tab. 3. Distribution of oestrogen receptors and androgen receptors in breast cancer tissues.

Receptors		Breast cancer tissue	
Oestrogen	Androgen	Primary* (%)	Metastatic** (%)
+	+	25.0	18.2
+	–	17.7	18.2
–	+	16.2	19.3
–	–	41.1	44.3

* n = 68
** n = 88

The results of CEA assays in extracts of benign and malignant tumours were classified according to probit analysis. Malignant tumours were plotted in different symbols, depending on their receptor positivity or receptor negativity. This procedure provided an impression of the distribution of receptor-positive and receptor-negative tumours over the various CEA content ranges of these tumours (figs. 2 and 3).

Figure 2 shows the results in 100 tumour extracts with reference to oestrogen receptors. The figure shows two groups with different standard distribution curves. The borderline CEA value seems to be approximately 1.5 μg/g protein. The group with CEA < 1.5 μg/g protein includes: all benign tumours, which are oestrogen receptor-negative (8,9) and 63.2% of the malignant tumours.

An evaluation of the distribution of oestrogen receptor-positive and oestrogen receptor-negative tumours within both groups, shows that 68.0% of the tumours in the group with CEA > 1.5 μg/g protein contained oestrogen receptors; in the group with CEA < 1.5 μg/g protein, only 32.5% of the malignant tumours contained oestrogen receptors.

Figure 3 shows the results of CEA assays in extracts in relation to androgen receptors in 90 tumours. Of the benign tumours (all with < 1.5 μg/g), 30% were androgen receptor-positive. The group with < 1.5 μg/g included 65.5% of the malignant tumours.

A relation between the presence of androgen receptor and the CEA level in extracts of malignant breast tumours was not demonstrable. The percentages of androgen receptor-positive malignant tumours in both groups (< 1.5 μg/g and > 1.5 μg/g) were virtually equal: 57.9 and 55, respectively.

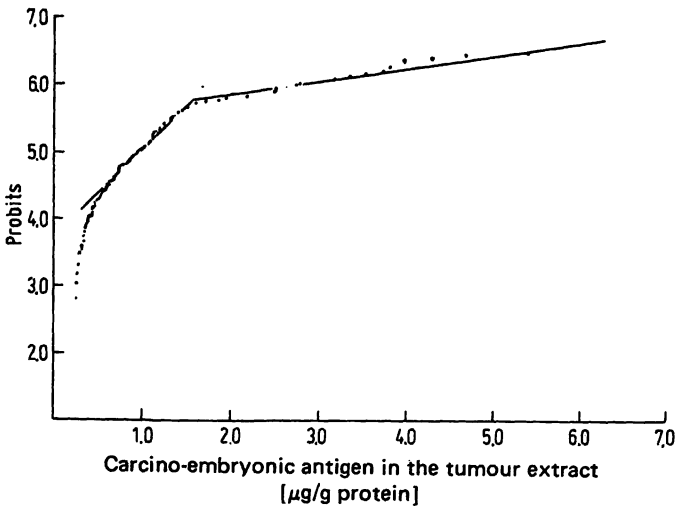

Fig. 2. Probit analysis of CEA quantities (μg/g protein) determined in benign (crosses) and malignant (circles) human breast tumours. The solid circles represent oestrogen receptor-positive tumours; the open circles oestrogen receptor-negative tumours. Two receptor-positive and two receptor-negative tumours with CEA content exceeding 7 μg/g are not inserted in the figure.

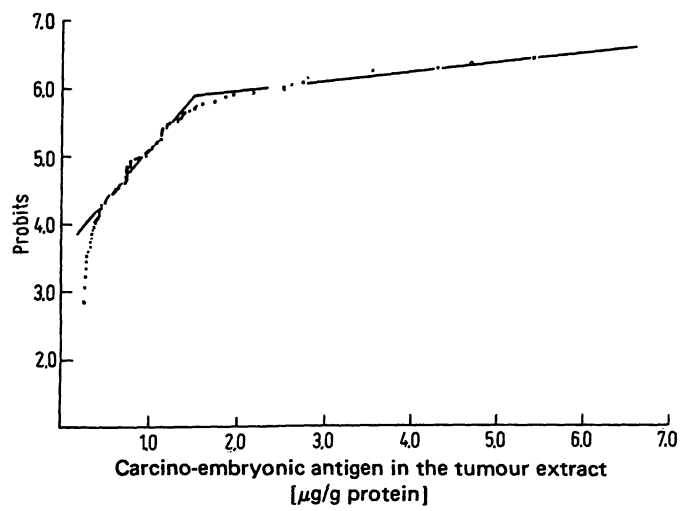

Fig. 3. Probit analysis of CEA quantities (μg/g protein) determined in benign (crosses) and malignant (circles) tumours. The solid circles represent androgen receptor-positive tumours; the open circles androgen receptor-negative tumours. Two receptor-positive and two receptor-negative tumours with CEA content exceeding 7 μg/g are not inserted in the figure.

Discussion

The data so far presented warrant three conclusions.

1. The oestrogen receptor assay cannot be replaced by the CEA assay in tumour extract.
2. Two groups of breast tumours can be distinguished: one of largely oestrogen receptor-negative tumours with a low CEA level, and one of largely oestrogen receptor-positive tumours containing larger amounts of CEA.

3. Breast tumours vary widely with respect to the presence of individual receptors, and the CEA level. In fact, all possible permutations of CEA, oestrogen receptor and androgen receptor can be encountered. There are tumours which contain only oestrogen receptors or only androgen receptors, or only CEA, and other tumours which contain some or all of these three substances, or none of them.

The oestrogen receptor is an unstable substance, and a considerable practical advantage would be gained if the oestrogen receptor assay performed with a view to therapeutic strategy in breast cancer patients could be replaced by an assay of a more stable substance, e.g. CEA. *Botet* et al. (5) cautiously concluded from their studies that this would be impossible. Our results provide strong evidence in favour of this conclusion. Figure 2 shows that definitely more than 10 percent of the oestrogen receptor-negative metastases can be regarded as CEA-positive. Without further clinical studies it can therefore be maintained that CEA assay in tumour extracts does not ensure a better selection of hormone-unresponsive patients than can now be achieved by means of the oestrogen receptor assay.

Better selection could perhaps be achieved by determination of more than one parameter. An indication to this effect can be found in a report by *Persijn* et al. (3) on androgen receptors in breast tumours. They supplied evidence that determination of androgen receptor combined with that of oestrogen receptor enhanced the prediction of the effect of ovariectomy. Extensive further clinical studies are required to establish whether CEA assays combined with assays of receptors or other ‚tumour markers' can significantly enhance the prediction of therapeutic effects in some cases.

The second conclusion calls for some qualification. Since about 90% of the oestrogen receptor-negative tumours are hormone-unresponsive[2]), the second conclusion could with some audacity be: hormone-unresponsive tumours have a significantly higher incidence of low CEA levels than hormone-responsive neoplasms. *Gold & Freedman* (11) tried to explain the appearance of CEA during carcinogenesis by postulating de-repression of previously repressed genetic information, leading to resumption of the synthesis of foetal antigens. They presented a detailed discussion of two possible mechanisms of de-repression (11).

In view of the hypothesis of *Gold & Freedman,* our finding could mean a relation between the hormone responsiveness of a tumour and the type and degree of de-repression underlying the development of the tumour.

If, on the other hand, we accept the theory of *Collins & Black* (12), who hold that CEA is also present in normal cells, as ‚cryptic' antigen, then our finding implies that hormone-responsive and hormone-unresponsive tumours arise from different cell types.

The above-mentioned relation between hormone responsiveness and CEA content assumes yet another dimension in view of the results presented in figure 3. This figure shows that there is no relation at all between the presence of CEA and that of androgen receptors in breast tumours. In this context we consider it of interest to note that mainly metastases in postmenopausal women were analysed in this study and that, in a previous report, we supplied evidence that androgen receptors are not a suitable parameter in predicting hormone responsiveness in postmenopausal women (3).

In summary, we feel justified in maintaining that our results may open a new avenue of understanding of the complex phenomenon of hormone responsiveness of some, and hormone unresponsiveness of other human breast tumours.

Further studies on the diversity of receptors, antigens or other 'tumour markers' in relation to therapeutic effects and the ending of remissions are required.

[2]) Clinically, a tumour is defined as hormone-responsive whenever hormonal therapy (be it additive or ablative) proves to be effective. This term must not be confused with the adjective ‚hormone-responsive' used in endocrinology with reference to precisely those tissues that for their growth depend on the presence of physiological concentrations of hormones in the blood.

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